DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
Food and Drug Administration

DEC 17 2003

Memorandum

Date:

From: Interdisciplinary Scientist/Pharmacist , Division of Dietary Supplement Programs , Office of Nutritional Products, Labeling and Dietary Supplements, HFS-810

Subject: 75-Day Premarket Notification of New Dietary Ingredients

To: Dockets Management Branch, HFA-305

Subject of the Notification:
7-hydroxymatairesinol (HMR) potassium acetate complex

Firm: Hormo Nutraceutical Oy Ltd.

Date Received by FDA: **3/19/03**

90-Day Date: **6/19/03**

In accordance with the requirements of section 413(a) of the Federal Food, Drug, and Cosmetic Act, the attached 75-day premarket notification and related correspondence for the aforementioned substance should be placed on public display in docket number 95S-0316 as soon possible since it is past the 90-day date. Thank you for your assistance.

Victoria Lutwak
For Gloria Chang

P drive/ NDI/ NDI File Closeout/DDSP SOP closeout process...

95S-0316

RPT 1&3

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

Food and Drug Administration
College Park, MD

MAY 23 2003

Lars Pellas
Chief Executive Officer
Hormos Nutraceutical Oy Ltd.
PharmaCity
Itainen Pitkakatu 4B
20520 Turku
Finland

Dear Mr. Pellas:

This is to inform you that the notification dated March 12, 2003, you submitted to pursuant to 21 U.S.C. 350b(a)(2)(section 413(a)(2) of the Federal Food, Drug, and Cosmetic Act (the Act) was filed by the Food and Drug Administration (FDA) on March 19, 2003. Your notification concerns the substance, 7-hydroxymatairesinol (HMR), which you intend to market as a new dietary ingredient. You state that HMR is extracted and purified from spruce tree (*Picea abies*) wood chips and further processed as hydroxymatairesinol potassium acetate complex (HMR-potassium acetate complex). The dosage form will be oral capsules containing approximately 7 to 150 mg of HMR or 10 to 125 mg of HMR-potassium acetate complex. The suggested condition of use is one capsule per day for a recommended maximum daily consumption of 150 mg HMR/day or approximately 3 mg/kg body weight for a 50 kg person and near 2 mg/kg body weight/day for a 70 kg person.

Under 21 U.S.C. 350b(a), the manufacturer or distributor of a dietary supplement that contains a new dietary ingredient that has not been present in the food supply as an article used for food in a form in which the food has not been chemically altered must submit to FDA, at least 75 days before the dietary ingredient is introduced or delivered for introduction into interstate commerce, information that is the basis on which the manufacturer or distributor has concluded that a dietary supplement containing such new dietary ingredient will reasonably be expected to be safe. FDA reviews this information to determine whether it provides an adequate basis for such a conclusion. Under section 350b(a)(2), there must be a history of use or other evidence of safety establishing that the new dietary ingredient, when used under the conditions recommended or suggested in the labeling of the dietary supplement, will reasonably be expected to be safe. If this requirement is not met, the dietary supplement is deemed to be adulterated under 21 U.S.C. 342(f)(1)(B) because there is inadequate information to provide reasonable assurance that the new dietary ingredient does not present a significant or unreasonable risk of illness or injury.

Federal regulations found at 21 CFR 190.6 specify the requirements for a pre-market notification on a new dietary ingredient. The notification you sent us concerning hydroxymatairesinol potassium acetate complex (HMR-potassium acetate complex) does not comply with the requirements of 21 CFR 190.6 and is incomplete. The Latin binomial name of the source of your product does not meet the requirements at 21 CFR 190.6(b)(2) which states that it should include the name of the new dietary ingredient that is the subject of the premarket notification, including the Latin binomial name (including the author) of any herb or other botanical. You referenced and summarized both published and unpublished *in vivo* and *in vitro* animal studies, mutagenicity studies, and human studies. In accordance with 21 CFR 190.6 (b) (4), any references or citations to published information offered in support of the notification shall be accompanied by reprints or photostatic copies of such references. If any part of the material submitted is in a foreign language, it shall be accompanied by an accurate and complete English translation. Further, no conclusions can be drawn from the brief summary statements and references to the unpublished studies. Information such as the investigator(s) name(s), credentials, affiliations, location and date when the unpublished studies were conducted and complete study reports are needed. You also did not provide adequate information on conditions of use under 21 CFR 190.6(b)(3)(ii).

Your notification will be kept confidential for 90 days after the filing date of March 19, 2003. After the 90-day date, the notification will be placed on public display at FDA's Docket Management Branch in docket number 95S-0316. Prior to that date, you may wish to identify in writing specifically what information you believe is proprietary, trade secret or otherwise confidential for FDA's consideration.

Should you have any questions concerning this matter, please contact Victoria Lutwak at (301) 436-1775.

Sincerely yours,

for

Susan J. Walker, M.D.
Acting Director
Division of Dietary Supplement Programs
Office of Nutritional Products, Labeling
and Dietary Supplements
Center for Food Safety
and Applied Nutrition

HORMOSnutraceutical

March 12, 2003

Office of Nutritional Products, Labeling, and Dietary supplements (HFS-820)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Pkwy
College Park, MD 20740

RE: New Dietary Ingredient Notification

Dear Sir or Madam:

Please find enclosed information pursuant to section 413(a) of the Federal Food, Drug and Cosmetic Act, in support of Hormos Nutraceutical Oy Ltd's marketing of the new dietary ingredient hydroxymatairesinol. Hormos Nutraceutical Oy Ltd intends to market this ingredient for dietary supplement use.

Sincerely,

Lars Pellas
Chief Executive Officer
Hormos Nutraceutical Oy Ltd.
PharmaCity
Itäinen Pitkäkatu 4 B
20520 Turku
FINLAND

In accordance with the Dietary Supplement Health and Education Act of 1994 (DSHEA), 21 U.S.C. § 350b (a) (2), and with final regulations published in Federal Register 49886-49892), 21 C.F.R. § 190.6 "Requirement for Premarket Notification", the following information is submitted by Hormos Nutraceutical Oy Ltd. in support of a New Dietary Ingredient Notification for hydroxymatairesinol. Hormos Nutraceutical Oy Ltd. intends to market hydroxymatairesinol as a dietary supplement in the United States. As per the statutes of the DSHEA, 21 U.S.C. § 350b (a) (2), Hormos Nutraceutical Oy Ltd. will not introduce, market, distribute or sell hydroxymatairesinol until at least 75 days following official acknowledgement of the receipt of this notification by the U.S. Food and Drug Agency (FDA).

SECTION 1

The name and complete address of the manufacturer of the dietary supplement that contains the dietary ingredient, or the dietary ingredient.

The manufacturer of the dietary ingredient will be:

Hormos Nutraceutical Oy Ltd.
Pharmacity
Itäinen Pitkäkatu 4C
FIN-20520 Turku
Finland

Attention: Mr. Lars Pellas
Vice President,
Corporate Development
Hormos Medical Corporation
Pharmacity
Itäinen Pitkäkatu 4C
FIN-20520 Turku
Finland

SECTION 2

The name of the dietary ingredient.

The new dietary ingredient is 7-hydroxymatairesinol (HMR), a dibenzylbutyrolactone plant lignan containing a 2,3-dibenzylbutane skeleton. Thus, HMR is chemically a close relative to matairesinol, a lignan present at high concentration in flaxeed. HMR is found at relatively high concentrations in the heartwood of branches and knots of spruce trees (*Picea abies*). In addition, detectable levels are present in sesame seeds. Hormos Nutraceutical Oy Ltd. intends to manufacture HMR as a potassium acetate complex. HMR is a near white powder. Because of the chiral carbon at position 7, HMR is composed of 2 stereoisomers (allo-HMR and HMR) that are present in an approximate 1:9 ratio within the HMR potassium acetate preparation. HMR and its potassium acetate complex are slightly soluble in water and soluble in acetone, aqueous ethanol, methanol, acetonitrile, DMSO, PEG, and corn oil.

HMR is extracted and purified from spruce tree wood chips. In the first phase of the process ground wood chips and ethanol are charged to a reaction vessel, heated to 75±4 °C and agitated for 10±4 hours. The mixture is then cooled to 50±5 °C, agitation stopped, and the wood chips left to settle. The ethanol solution is separated from the wood chips by filtration and an additional 500±80 kg of ethanol is then added to the reactor (still containing wood chips) and the solution heated to 75±4 °C and agitated for 5±1 hours. The ethanol solution is again separated from the wood chips by filtration and then added to the ethanol solution derived from the first extraction. The combined ethanol solutions are concentrated with a falling-film evaporator. The distillation residue is then packed, weighed, and analyzed.

The second phase of the manufacturing process involves the crystallization of HMR from the concentrated extract as a potassium acetate complex. A 120 kg ethanol solution containing 23.4 kg of HMR distillation residue is charged to an enamel-lined reactor along with 54±1 kg of pure ethanol, 99±1 kg of ethyl acetate, 5.8±0.2 kg of water, and 2.9±0.2 kg of acetic acid. The temperature of the reactor is adjusted to 25±3 °C and 20±1 kg of potassium acetate is added. This mixture is then agitated for 40±20 minutes and seeded with 10±5 grams of previously manufactured HMR potassium acetate complex. Agitation is continued for 16±8 hours at 25±3 °C and the product allowed to crystallize. The crystallized product is then centrifuged and washed with a pre-cooled mixture of ethyl acetate and ethanol. The resulting product is then dried under vacuum at 48±5 °C, cooled to 30±5 °C, and then packed, weighed, and analyzed.

SECTION 3

Description of the dietary supplement or dietary supplements that contain the dietary ingredient including (i) the level of dietary ingredient in the dietary supplement, and (ii) the conditions of use recommended or suggested in the labeling of the dietary supplement, or if no conditions of use are recommended or suggested in the labeling of the dietary supplement, the ordinary conditions of use of the supplement.

Hydroxymatairesinol potassium acetate complex is expected to be manufactured in a form that is greater than 95% pure. HMR will be sold in the form of oral capsules containing approximately 7 to 150 mg of active ingredient (*i.e.*, HMR). Since potassium acetate, the complexing salt, accounts for 20 to 30% of the active ingredient formulation on w/w basis, the total amount of HMR-potassium acetate complex in each capsule will be up to 10 to 215 mg, respectively. Consumption of 1 capsule per day will be suggested or recommended, resulting in a recommended maximum daily consumption of 150 mg HMR/day, or approximately 3 mg/kg body weight for a 50 kg person and near 2 mg/kg body weight/day for a 70 kg person.

SECTION 4

The history of use or other evidence of safety establishing that the dietary ingredient, when used under the conditions recommended or suggested in the labeling of the dietary supplement, will reasonably be expected to be safe, including any citation to published articles or other evidence that is the basis on which the distributor or manufacturer has concluded that the dietary supplement will reasonably be expected to be safe.

The overall safety HMR is supported by the results of pharmacology, toxicology and related testing in animals, clinical studies in humans, and by data on other related plant lignans that are consumed directly in conventional food or which are marketed as dietary supplements.

Animal Studies

The results of unpublished standard general pharmacology studies demonstrate that HMR does not have pharmacological activity. Specifically, HMR did not affect mean arterial pressure and heart rate in rats administered doses of up to 30 mg/kg body weight by i.v. injection. There were no effects of HMR on pentobarbital-induced sleeping times in rats dosed by oral gavage at up to 100 mg/kg body weight. Similarly, at this dose level HMR did not alter motor co-ordination, spontaneous motor activity, and gastrointestinal transit time in mice, or rectal temperatures, pain thresholds, and kidney function in rats. There were no overt effects of HMR, administered orally at doses of up to 1,000 mg/kg body weight, in male NMRI mice in the Modified Irwin Screen test. In telemetered dogs treated by oral gavage with single HMR doses of 2, 20, and 200 mg/kg body weight, there were no obvious effects on respiratory rate, tidal volume, or minute volume. Electrocardiograms showed no effects of HMR on P-wave amplitude, P-wave duration, P-Q interval, QRS interval, or Q-T interval.

The toxicity of HMR has been extensively studied in animals. One acute toxicity study, 3 subchronic toxicity studies (14-, 28-, and 90-day studies), and a teratology study have been performed in rats. A 14-day and a 28-day study have also been conducted in beagle dogs.

In an unpublished acute toxicity study, single gavage doses of 500, 1,000, and 2,000 mg HMR/kg body weight were administered to groups of male and female rats. Signs of toxicity were then monitored for 14 days. The test was performed in accordance with Organization for Economic Co-operation and Development (OECD) guidelines for acute oral toxicity testing. There were no deaths or other signs of toxicity reported. Similarly, there was no effect of treatment on macroscopic or microscopic observations. The authors concluded that the minimal lethal dose was above 2,000 mg HMR/kg body weight.

In the 28-day subchronic rat study, HMR was administered by oral gavage to groups of 10 male and 10 female Sprague-Dawley rats at doses of 0, 239, 477, and 955 mg/kg body weight/day, and 0, 242, 483, and 967 mg/kg body weight/day for the first 18 and the second 15 days, respectively. An additional 3 animals/sex/group were included for toxicokinetic analyses. There were no treatment-related effects on body weight, body weight gains, food consumption, organ weights, or on hematological, clinical chemistry, and urinalysis parameters. Four high-dose females, 1 high-dose male, and 1 mid-dose female died likely due to a relatively large volume of a bolus dose combined with a thick consistency of the test article formulation. There was no effect of treatment on the incidence of findings at pathological examination, except for the finding of a large thyroid in 1 high-dose male. Histopathological examination indicated the presence of slight follicular cell hypertrophy in high-dose males. The relationship of this finding to treatment was unknown; however, since no indications of thyroid follicular cell hypertrophy were seen in a 90-day study in which rats were treated with HMR at dose levels of up to 2,600 mg/kg body weight/day, the finding in the 28-day study was considered incidental. In any case, oral gavage administration of HMR at doses of up to 967 mg/kg body weight/day for 28 days was well tolerated without overt toxicity.

In a preliminary "Maximum Tolerated Dose" study, HMR was in capsule form to 1 male and 1 female beagle dog at escalating doses of 0, 200 (days 1-4), 500 (days 5-7), and 700 (days 8-11) mg/kg body weight/day for 11 days. At the end of dosing, the control animals were dosed at 700 mg/kg body weight/day for 15 days. A second formulation of HMR was tested in capsule form at a dose of 636 mg/kg body weight/day for 5 days in one female and one male dog used in the MTD phase of the study. The MTD phase of the study demonstrated that 700 mg/kg body weight/day was well tolerated. Dosing at 700 mg/kg body weight/day for 15 days produced no treatment-related effects on clinical signs, mortality, body weight, body weight gain, food consumption, hematology or clinical chemistry parameters, or on the result of organ weight assessment and macroscopic examinations.

HMR was subsequently tested in groups of 3 male and 3 female beagle dogs by daily administration in capsule form at doses of 0, 146-149, 341-347, and 682-685 mg/kg body weight/day for 28 days. There were no treatment-related effects on mortality, clinical signs, hematological or clinical chemistry parameters, urine analysis, organ weight data, or on the results of electrocardiographic examinations. The group mean weight of the high-dose males was found to be consistently lower than controls at pre-dose and throughout the study period. Similarly, in high-dose animals, food consumption appeared slightly decreased compared to controls. Food consumption did increase in these groups as the study progressed. The mean absolute and relative uterine weights appeared increased in dosed females; however, given the lack of dose-response or of histopathological correlates, and noting that all of the values were within the historical control ranges, this finding was not considered to be related to HMR treatment. Histopathological examination revealed a single healing erosion in the ileum of 1 high-dose female dog (correlating to a red, depressed area noted at gross necropsy) and of follicular cell hypertrophy in 1 male and 1 female of the high-dose groups. Due to the small number of animals used in this study, the relationship of these macroscopic and histopathological findings cannot be ascribed to treatment, but neither can a treatment-related effect be discounted.

In an unpublished subchronic toxicity study, groups of 20 male and 20 female Wistar rats were fed HMR in the diet at concentrations of 0, 0.25%, 1.0%, and 4.0% for 13-weeks. These dietary concentrations resulted in HMR intakes of approximately 0, 160, 640, and 2,600 mg/kg body weight/day. There were no effects of HMR treatment on mortality, neurobehavioral observations, motor assessment results, ophthalmoscopic examinations, urinalysis parameters, sperm analysis, or on the results of the macroscopic examinations. Histopathological examination revealed a decreased incidence of hyaline droplet nephropathy, a beneficial effect, in high-dose males.

Male rats dosed at 4.0% in the diet showed decreased body weights throughout the study period. In mid-dose males and in mid- and high-dose females, this effect occurred only in the first few weeks of the study. In both sexes treated at the high-dose, there was an increase in the number of animals with sparsely haired skin. Hematological analyses at the end of the study revealed increased thrombocytes in high-dose females and increased WBC count and absolute neutrophil count in high-dose males. Several clinical chemistry changes were noted, including: decreased cholesterol and fasting glucose in high-dose males, increased albumin and A/G ratio in high-dose males, increased GGT in high-dose females, decreased triglycerides in treated males, decreased phospholipids in mid- and high-dose males, and increased total bilirubin in mid- and high-dose males. The clinical chemistry changes, in particular the findings of reduced cholesterol, triglycerides, and phospholipids, have been noted in response to dietary administration of high fiber concentrations that are metabolized by gut microflora (as with HMR). This metabolism results in production of short-chain fatty acids that stimulate hepatic and peripheral metabolism of carbohydrates and fats (Roberfroid, 1993; Roberfroid and Slavin, 2000; Adam *et al.*, 2001).

Several organ weight changes were reported in the treated groups. In treated males and in high-dose females, the relative weight of the filled cecum was increased. Similarly, both the absolute and relative weights of the empty cecum were increased in males at the top 2 dose levels and in high-dose females. This effect is a well-known physiological response to high-dietary concentrations of certain substances that alter the osmotic loading of the lower gut, and as such is not considered adverse (DeGroot *et al.*, 1974; WHO, 1987; Scheppach, 1994; Lynch *et al.*, 1996; Adam *et al.*, 2001). In high-dose males, the relative kidney and testes weights were increased, and the relative weight of the adrenal and the absolute brain weight were decreased, in comparison to controls. Modest decreases (about 10%) in ovarian weights were reported in treated females, with the relative ovarian weight decreased in mid- and high-dose groups. There were no histopathological correlates for any of the reported organ weight changes.

Vaginal smears taken during the last 3 weeks prior to sacrifice demonstrated that the number of high-dose females with a maximum estrous cycle length of 4 days was decreased, while those with a maximum cycle length of 5 days was increased, in comparison to controls. This finding was also reflected in an increased mean estrous cycle length in this dose group.

The effects of HMR on ovarian weights and on estrous cycle length could be indicative of a very weak antiestrogenic effect consistent with stronger such effects known for many other phytoestrogens, including soy isoflavones and secoisolariciresinol diglycoside (SDG) derived from flaxseed (Orcheson *et al.*, 1998; Tou *et al.*, 1998, 1999). These effects may be related to the metabolism of HMR to enterolactone (Orcheson *et al.*, 1998). Minor changes in ovarian weights and in estrous cycle length are not adverse and are not indicative of strong estrogenic potency. This is evidenced by the lack of estrogen receptor binding of HMR in an *in vitro* study. The study authors reported a "No Observable Effect Level" (NOEL) of 0.25% in the diet, or, for Wistar rats, a dose of approximately 160 mg/kg body weight/day.

The teratogenic potential of HMR has been evaluated in an unpublished prenatal developmental toxicity study. HMR was administered in the diet to groups of 24 mated female Wistar out bred rats at concentrations of 0, 0.25%, 1.0%, and 4.0% from gestational day 0 (fertilization) through until gestational day 21. These dietary concentrations equated to HMR intakes of approximately 0, 140-180, 460-740, and 1,190-2,930 mg/kg body weight/day in the control through high-dose groups, respectively. At Caesarean section dams and fetuses were macroscopically examined and the fetuses, placental material, reproductive organs, and the full and empty cecum weighed. Fetuses were further evaluated for both visceral and skeletal abnormalities.

Thirteen of the high-dose animals (out of 24) were sacrificed due to the fact that they either did not eat or ate less than 4 g of food per day. There were no differences in the clinical signs between the treated and control groups. Both body weight and food consumption were significantly decreased in high-dose animals from gestational day 3 through until Caesarean section. This was most probably related to poor palatability of the diet. Necropsy of the dams did not reveal any treatment-related pathological changes. The absolute and relative weights of the full cecum were increased in the high-dose group while the absolute and relative weights of the empty cecum were increased in both the mid- and high-dose groups. There were no pathological changes associated with the increased cecal weights. The study authors concluded that the maternal NOEL was at 1.0% in the diet or at least 460 mg/kg body weight/day based on a decreased body weight at the top dose.

There were no effects of treatment on reproductive indices including female fecundity, number of corpora leutea, implantation sites, number of live fetuses, number of early and late resorptions, sex-ratio, and amount of pre- and post-implantation loss. External evaluation of the fetuses and placental material revealed no effects of treatment. The weights of the fetal females and of all fetuses combined were decreased from dams of the high-dose group. There were no treatment-related effects on the incidence of either visceral or skeletal abnormalities. The incidence of kinked ureter was increased in fetuses from the mid- and high-dose dams; however, this particular finding is generally considered a variation (Brown, unpublished, 2002), and essentially harmless (Chahoud *et al.*, 1999). As a result, the study authors concluded that the NOEL for developmental toxicity was at least 4.0% in the diet (at least 1,190 mg/kg body weight/day), the highest dose tested in the study.

The genotoxicity of HMR was evaluated in 3 unpublished studies sponsored by Hormos Nutraceutical Oy Ltd., including an Ames bacterial mutagenicity assay, an *in vitro* chromosome aberration assay, and an *in vivo* rat bone marrow micronucleus study. All 3 of these studies were conducted according to OECD protocols and were consistent with U.S. FDA Redbook guidelines.

In the Ames assay, the test substance in dimethylsulfoxide (DMSO) was not toxic to *Salmonella typhimurium* strain TA100 at concentration levels of 1.6, 8, 40, 200, 1,000, or 5,000 μg/plate. Similarly, HMR was not toxic to *Salmonella typhimurium* strains TA98, TA102, TA1535, or TA1537 at concentrations of 5,000 μg/plate. Following incubation with HMR, both in the absence and in the presence of an exogenous source of metabolic activation (Aroclor 1254-induced liver post-mitochondrial fraction (S9) from Sprague-Dawley rats), there were no biologically or statistically significant increases in the number of revertant colonies in comparison to solvent treated controls.

HMR was tested in an *in vitro* cytogenetic assay using Chinese hamster ovary cells. In one set of experiments, HMR was incubated, either in the presence or absence of an exogenous source of metabolic activation (rat S9), for 3 hours followed by a 17-hour recovery period. Concentrations tested ranged from 0 to 1,083 μg/ml (without S9) and from 0 to 1,227 μg/ml (with S9). At the highest concentrations tested in each case there was a significant increase in the number of cells with chromosomal aberrations compared to solvent treated controls. However, at these concentrations, cell numbers were decreased by 51 and 59% in the presence and absence of S9, respectively. This indicates that concentrations associated with chromosomal aberrations were also associated with significant cytotoxicity. In a second series of experiments, treatment was repeated in the absence and in the presence of S9 for 3 hours plus a 17-hour recovery period. As in the first experiment, the highest concentrations tested (862.4 μg/ml and 1,253 μg/ml in the absence and presence of S9, respectively) were associated with an increase in the frequencies of structural aberrations. However, as before, at these concentrations, a 51 to 59% reduction in cell survival was reported. As a result, the study authors concluded that HMR could induce chromosome aberrations, but only at concentrations inducing at least 50% cytotoxicity.

In the *in vivo* rat bone marrow micronucleus assay, groups of 6 male Charles River CD rats were treated with HMR by corn oil gavage at doses of 0, 500, 1,000 and 2,000 mg/kg body weight once daily for 2 consecutive days. Doses were established on the basis of an initial toxicity study in 3 male and 3 female rats. The rats were killed 24 hours after the second administration. Treatment with HMR did not increase the group mean ratios of polychromatic:normochromatic erythrocytes above the values reported for the negative controls or in comparison to historical control ranges. Similarly, the frequencies of micronucleated polychromatic erythrocytes were unaffected by treatment. The positive control, cyclophosphamide, administered by gavage in saline at a dose of 20 mg/kg (single dose) produced the expected increase in micronucleated polychromatic erythrocytes. Based on these results, the study authors concluded that HMR did not induce genotoxic effects in this assay system. These results also support the conclusion that chromosomal aberrations reported in the *in vitro* CHO study are solely due to cytotoxic effects.

Taken together, the results of the 3 genetic toxicity studies indicate that HMR is without intrinsic genotoxic potential.

The metabolism of HMR was evaluated in a published study (Saarinen[1]) in rats. Rats were administered HMR by oral gavage at doses of 3, 15, 25, and 50 mg/kg body weight on 2 consecutive days during which time they were housed in metabolic cages and urine collected for HPLC analysis of metabolites. Enterolactone was the major metabolite identified reaching levels in the urine that were more than 8-fold those found in controls. Other metabolites detected included hydroxyenterolactone, α-conidendrin, conidendric acid, enterodiol, allo-HMR, and unchanged HMR. No analysis of potential fecal metabolites was conducted. All of these minor metabolites, including unchanged HMR, were at concentrations many-fold (usually greater than 10-fold) lower than the primary metabolite enterolactone.

In another unpublished study designed to evaluate the absorption and tissue distribution of HMR, tritiated-HMR was administered to groups of 2 male and 2 female Sprague – Dawley rats by both oral gavage and intravenous injection. Following oral administration at 250 mg/kg body weight, measures of total radioactivity indicated that HMR was well absorbed, with peak plasma concentrations occurring approximately 1 hour post-dosing. Non-volatile radioactivity was reported to be well distributed to body tissues with the highest concentrations found in the gastrointestinal and urinary systems. Rapid clearance of total radioactivity (concentrations in tissues and plasma less than 10% of peak values at 24 hours post-dose) was reported, indicative of rapid excretion in the urine. By comparing AUC values for plasma concentrations from the oral study with AUC values obtained following dosing *via* intravenous injection (25 mg/kg body weight), the authors concluded that about 56% of the initial radiolabeled dose was absorbed following oral administration. In addition, it was apparent that the sites of the radiolabel in parent HMR were stable as there was very minimal exchange of radiolabel with body water.

The results of the *in vivo* metabolic studies are complimented by an *in vitro* study in which HMR, as the individual diastereoisomers HMR and allo-HMR, was incubated in the presence of human or rat liver homogenate preparations. Both HMR and allo-HMR were rapidly metabolized by the human liver homogenate (*i.e.*, about 80%-90% cleared

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[1] Saarinen NM, Wärri A, Mäkelä S, Eckerman C, Reunanen M, Ahotupa M, Salmi SM, Franke AA, Kangas L and Santti R. (2000) Hydroxymatairesinol, a novel enterolactone precursor with antitumor properties from coniferous tree (Picea Abies), Nutr. Cancer., 36, 207-216.

within 60 minutes), with glucuronidation comprising the most significant biotransformation process, accounting for more than 95% of the metabolites generated. Compared to the human liver homogenate, rat liver preparations were much less efficient in clearing either HMR isomer. Only about 30%-60% was cleared within 60 minutes by the rat liver preparation. The metabolite profile and the active biotransformation processes, however, were similar between the 2 species. In contrast to the results of the *in vivo* study in rats, enterodiol and enterolactone metabolites were not found to occur following incubation with either the human or rat liver homogenate *in vitro*. This result is expected given the recognised conversion of plant lignans into their mammalian counterparts (enterolactone and enterodiol) by way of facultative intestinal microbes (Axelson et al[2]).

In addition to the preceding standard pre-clinical studies, Hormos Nutraceutical Oy Ltd. sponsored several specialized studies to investigate the antioxidant, chemo-preventative, and potential estrogenic activity of HMR.

The results of the unpublished investigations of the antioxidant properties of HMR, both *in vitro* and *in vivo*, showed that this substance was an effective antioxidant. *In vitro*, HMR: a) inhibited lipid peroxidation induced by tert-butylhydroxyperoxide (IC_{50} of 0.06 µmol/L), b) inhibited oxidation of human low-density lipoprotein (LDL) by copper (IC_{50} of 6.7 nmol/mg LDL), c) inhibited LDL incorporation in serum (IC_{50} of 130 nmol/mg LDL), d) scavenged superoxide anions produced by xanthine-xanthine oxidase (EC_{50} of 5.6 µmol/L), and e) scavenged peroxyl radicals generated by thermal decomposition of 2,2'-azobis(2-amidinopropane) hydrochloride (1 mole of peroxyl radical scavenged per 4 moles of HMR). Minor metabolites of HMR, including α-conidendrin and conidendric acid also showed significant antioxidant activity in the above *in vitro* assays.

In one of the *in vivo* antioxidant studies, weanling male Sprague-Dawley rats were fed a diet containing HMR to provide a dose of approximately 40 mg/kg body weight/day for 5 months. Controls received diets not supplemented with HMR. Following the 5 month feeding period the animals were killed and liver, lung, and kidney tissues examined for signs of oxidative stress, specifically diene conjugation as an indication of lipid peroxidation. HMR had no effect on any measures of oxidative stress. In a second experiment, oxidative stress was induced experimentally in male mice through combined vitamin-E deficient diets and administration of carbon tetrachloride. Test diets contained either HMR (300 ppm or about 50 mg/kg body weight/day) or HMR in combination with α-tocopherol (restored to normal dietary levels). Diets were administered for up to 4 weeks. Oxidative stress was measured through analysis of the presence of thiobarbituate reactive substances (TBARS) in the liver. HMR alone and in combination was shown to substantially reduce the generation of TBARS (signs of oxidative stress). Also, HMR appeared to potentiate the antioxidant effects of vitamin E.

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[2] Axelson M., Sjovall J., Gustafsson BE and Setchell KD (1982) Origin of lignan in mammals and identification of precursor from plants. Nature 298, 659-660.
Axelson M and Setchell KD (1981) The excretion of lignans in rats – evidence for an intestinal bacterial source for this new group of compounds. FEBS lett. 123, 337-342.

In a published study of the chemo-preventative effects of HMR (Oikarinen *et al.*, 2000), the inhibitory effects of HMR or rye bran on intestinal tumor development was tested in in adenomatous polyposis colimultiple intestinal neoplasia $(Apc)^{Min}$ mice. HMR, along with inulin (2.5%), was administered to the male mice in a high fat diet, at a concentration of 200 ppm for a period of 5 to 6 weeks. Compared to the control diet (2.5% inulin, high fat diet), and compared to the control diet/rye bran combination, in HMR treated mice the mean number of adenomas in the small intestine was significantly lower (26.6 *versus* 39.6 and 36.0 in mice administered the control diet and the rye bran/control diet, respectively). HMR also appeared to normalize the levels of β-catenin concentrations within the adenomatous tissue. This was considered by the authors to indicate that the chemo-preventative effect of HMR was mediated through the Apc- β-catenin pathway. The authors did note that although there were no differences in body weight gain amongst the treatment and control groups, mice administered diet supplemented with HMR tended to have an increased amount of hair shedding.

Further chemo-preventative activity of HMR was demonstrated in an unpublished study in which, 3-days following injection of LNCaP prostate cancer cells into both flanks of male nude mice, treatment with HMR in the diet at concentrations of 0.15% and 0.3% for 9 weeks reportedly decreased mean tumor weight and serum concentrations of PSA. Similarly, in a rat mammary tumor model in which mammary tumors were induced by 7,12-dimethylbenz[a]anthracene, treatment with HMR in the diet was reported to inhibit tumor growth at doses in the range of 2 to 20 mg/kg body weight/day over a period of up to 17 weeks.

The potential estrogenic effects of HMR were evaluated in an unpublished study on the ability of HMR to competitively inhibit the *in vitro* binding of a fluorescein labeled estrogen receptor ligand (ES2) to estrogen receptors α and β. Competitive binding was compared to the standard of 17β-estradiol. HMR was found not to competitively bind with ES2 for either estrogen receptors α or β. The maximum concentration of HMR tested in this assay was 10,000 nM.

<u>Human Data</u>

In addition to the aforementioned preclinical safety data, 2 clinical trials have, or are, being conducted to assess the tolerability, safety, and toxicokinetics of HMR in human subjects.

The results of one clinical trial have been presented in an unpublished report. In this study, single doses (3, 10, 30, 100, 300, 600, and 1,200 mg) of HMR, or a placebo, were given to healthy male volunteers in capsule form. At the 4 lower doses levels (up to and including 100 mg), 3 subjects received HMR and 1 subject received placebo. At the 300 mg dose level, 8 subjects received HMR and 2 subjects received placebo. At the 2 highest dose levels, 5 subjects received HMR and 1 subject received the placebo. Plasma concentrations of HMR, enterolactone, and other minor metabolites of HMR were measured 7, 3, and 1 day prior to HMR/placebo administration to establish baseline values. Following consumption of the capsule, blood was collected for analysis at 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 30, 48, and 72 hours post-dosing. Similarly, urine was collected in fractions for the 3 day period following test article administration and analyzed for the presence of HMR and its conjugated and unconjugated metabolites. LDL and DNA oxidation were measured prior to treatment and 2, 6, and 48 hours post-dosing. Safety was monitored through physical examination, clinical chemistry and hematological measurements, ECG recordings, and adverse event reporting (if any).

The pharmacokinetic analysis revealed that peak concentrations of HMR in plasma were reached 0.5 to 2.0 hours post-dosing, regardless of the dose administered. This result is consistent with the pharmacokinetic data obtained in rats. C_{max} values ranged from 0.29 ng/ml at the 3 mg dose level to 326.86 ng/ml at the 1,200 mg dose level. While the plasma levels of HMR increased with dose, the increase was often not linear and showed considerable inter-individual variation. Also, concentrations of HMR were detected in the plasma of the placebo group, and, in some cases, these concentrations exceeded those associated with the low dose of 3 mg. Elimination of HMR from plasma was rapid with a $t_{1/2}$ values ranging from 2.6 to 5.1 hours.

With respect to safety, the study authors concluded that HMR was well tolerated without any report of serious adverse events. There was no effect of HMR on clinical chemistry and hematological evaluations, vital signs, ECG recordings, or on the results of the physical examinations. There were no clear beneficial effects of HMR treatment on LDL oxidation products or the oxidation of DNA as measured by the levels of 8-OH-guanidine.

A second tolerability, safety and toxicokinetic study has been completed, but not reported, involving multiple dosing of male volunteers with mild hypercholesterolemia. Also, this study was designed to investigate potential beneficial effects of HMR consumption on lipoprotein oxidation products in this study group.

In addition to the clinical studies on HMR, its safety is further corroborated by the history of safe consumption of plant lignans in the diet. Lignans possessing the 2,3-dibenzylbutane carbon skeleton (as with HMR) are ubiquitous in the fiber portion of higher plants. Plant lignans occur in most of the plant parts including roots, leaves, stem, seed, and fruits. As with HMR, intestinal metabolites of these higher plant lignans include enterolactone and enterodiol, 2 lignans that are present naturally in the blood of mammalian species, including humans. A number of plant-derived lignans and similar products (*e.g.*, soy isoflavones) are currently marketed as Dietary Supplements in the United States and have not been associated with the occurrence of adverse effects. Beyond the history of safe consumption of similar plant lignans in the normal diet, there is considerable evidence to suggest that consumption of plant lignans may have antioxidant and chemo-preventative effects. In particular, lignan rich diets are known to elevate enterolactone concentrations in the serum. Reduced enterolactone levels have been associated with increased risk for the development of breast cancer and the occurrence of acute cardiac events.

Based on the preceding information, the use of the new dietary ingredient HMR according to suggested labeling (*i.e.*, maximum daily intakes equivalent to 2 and 3 mg/kg body weight in a 70 and a 50 kg individual, respectively) is concluded to be safe.

RECEIVED
JUN 09 2003
BY:

June 3, 2003

Susan J. Walker, M.D.
Acting Director
Division of Dietary Supplement Programs
Office of Nutritional Products, Labeling, and Dietary supplements (HFS-820)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Pkwy
College Park, MD 20740
USA

Subject: Our New Dietary Ingredient Notification for 7-hydroxymatairesinol potassium acetate complex, filed by the FDA on March 19, 2003.

Dear Dr. Walker,

I refer to your letter dated May 23 informing us that our New Dietary Supplement Notification for 7-hydroxymatairesinol potassium acetate complex will be placed on public display at FDA's Docket Management Branch in docket 95S-0316 after a 90 days confidentiality period following the filing date of March 19, 2003.

Hormos Nutraceutical Oy Ltd. has made significant investments in the development of the manufacturing process for the 7-hydroxymatairesinol potassium acetate complex. The process is described in Section 2 of our notification. We consider this information to be our trade secret and confidential and we respectfully ask that the process description is deleted from the notification which will be made publicly available. I enclose a proposed public version of Section 2 of our notification for FDA's consideration.

We shall revert to the other questions raised in your letter shortly.

Yours sincerely,

Lars Pellas
Chief Executive Officer
Hormos Nutraceutical Oy Ltd.
PharmaCity
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SECTION 2

The name of the dietary ingredient.

The new dietary ingredient is 7-hydroxymatairesinol (HMR), a dibenzylbutyrolactone plant lignan containing a 2,3-dibenzylbutane skeleton. Thus, HMR is chemically a close relative to matairesinol, a lignan present at high concentration in flaxeed. HMR is found at relatively high concentrations in the heartwood of branches and knots of spruce trees (*Picea abies*). In addition, detectable levels are present in sesame seeds. Hormos Nutraceutical Oy Ltd. intends to manufacture HMR as a potassium acetate complex. HMR is a near white powder. Because of the chiral carbon at position 7, HMR is composed of 2 stereoisomers (allo-HMR and HMR) that are present in an approximate 1:9 ratio within the HMR potassium acetate preparation. HMR and its potassium acetate complex are slightly soluble in water and soluble in acetone, aqueous ethanol, methanol, acetonitrile, DMSO, PEG, and corn oil.

HMR is extracted and purified from spruce tree wood chips.